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*the Medical Center* / DEPARTMENT OF MICROBIOLOGY / September 23, 1978

Dr. Donald S. Fredrickson  
Director  
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Dear Don:

I have read the Proposed Revised Guidelines for Recombinant DNA Research and accessory information as published in the Federal Register on July 28, 1978 (p.33042-33178). Overall I am impressed with the way NIH has considered all evidence, opinions and other relevant information in drafting a well organized, comprehensive and realistic set of Guidelines to allow the substantial benefits now arising from this research to be attained for the benefit of all. In arranging my comments on the Proposed Revisions of the Guidelines for Recombinant DNA Research, I decided to consider each section of the Guidelines in order of appearance and to intermix my comments of support for certain sections with those seeking clarification of apparent ambiguity as well as those dealing with several minor and two more substantial criticisms of the Guidelines as now proposed. These two more significant criticisms concern Appendix A which lists microorganisms that exchange genetic information and the levels of containment for some experiments involving cloning of eukaryotic viral DNA sequences in EK1 host-vector systems of Escherichia coli K-12.

My comments are as follows:

1. The basis for the conclusion that recombinant DNA research with the E. coli K-12 host-vector systems is much safer than originally believed and the means by which this conclusion was reached are presented in a complete and thorough manner in the Decision of the Director to Issue Revised Guidelines and in the Proposed Environmental Impact Statement. I agree with this conclusion that was initially stated in my letter to you of April 22, 1977 and which I have reiterated on numerous more recent occasions. However, in view of the substantial data to support this conclusion, I was somewhat bothered to read statements such as "....no evidence has come to light of a product created by these techniques that has been harmful to man or the environment" (p. 33044, Col. 1) with the implication that these negative results are indicative of the safety of recombinant DNA research. I have criticized the use of this particular argument in behalf of the safety issue in a paper presented June 6, 1978 at the Third International Symposium on the Genetics of Industrial Microorganisms in Madison, Wisconsin. A preprint copy of this paper, which will soon be published in ASM News as well as in the Proceedings of the Symposium, is attached as Appendix A to this letter. In it I state "The sixth factor [leading to the general belief that recombinant research with E. coli K-12 host-vector systems is safer than originally believed]

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has been the absence of any noticeable biohazard associated with the construction of any recombinant during the past several years. While reassuring, it should be noted that biohazards were not looked for and, indeed, all experiments were conducted under levels of physical and biological containment that we now agree were over-restrictive and which would preclude manifestation and/or detection of any biohazard should they have arisen. Thus it is scientifically invalid to use the experience of five years' research as a justification to argue that biohazards do not exist. It will thus be necessary to design and conduct experiments expressly for the purpose of determining whether the introduction of foreign DNA does or does not result in manifestation of biohazards and such experiments have not been done". I should hasten to add that such experiments have commenced but have not been fully reported. I would, therefore, prefer that you downgrade the importance of the above-cited argument for the safety of recombinant DNA research and rely instead on the substantial body of factual information and valid experimentation to substantiate the basis for the conclusion that recombinant DNA research with E. coli K-12 host-vectors is far safer than any of us had originally believed four years ago.

2. Section I. Scope of the Guidelines (p.33069-33070). The organization and clarity of this section has vastly improved over both the previous Guidelines and the draft arrived at by the RAC. I do, however, have some specific comments on Sections I-D Prohibitions and I-E Exemptions as described in the following four points.

3. Sections I-D-1 and I-D-2 (p.33070). Since VSV and moderate risk oncogenic viruses are or can be construed to be Class 3 agents, there is a paradox which, while dealt with in the Decision of the Director to Issue Revised Guidelines statement, still persists in the Revised Guidelines as now written. It should be stated explicitly in Section V, footnote 2 (p.33086) that the basis for allowing cloning of genetic information from VSV and moderate risk oncogenic viruses is the likely difficulty of faithful transcription and/or translation of this genetic information in prokaryotic host-vector systems, which would therefore be less likely to lead to a biohazardous condition than might cloning genetic information from Class 3 prokaryotic or lower eukaryotic organisms. I might add that I hope that, in the near future, NIH would thoroughly consider the basis for prohibitions I-D-1 and I-D-2 in light of the fact that recombinant DNA research with organisms in the Class 3 category as well as the deliberate cloning of genes specifying potent toxins can provide information not now available on the biochemical and genetic bases of pathogenicity of some of these agents and can also provide information that might lead to effective means of diagnosis, treatment and/or prevention of diseases caused by these agents. Such work is definitely in the national interest and means for its conduct and performance should be facilitated. I thus strongly believe that the lifting of these two prohibitions should not be decided solely on a case-by-case basis but rather should be based on a general consideration of the benefits and risks of any and all experiments in either of these two categories. The facts that the allowed cloning of genetic information from some Class 3 viral agents already constitutes an exception to Section I-D-1 and the existence of P4 containment and the eventual approval of EK3 host-vector systems whose uses are not stipulated for any experiments in the Proposed Revised Guidelines should serve to justify

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reconsideration of all experiments now prohibited under Sections I-D-1 and I-D-2.

4. Section I-D-3. (p.33070). I do not understand why this prohibition is restricted to the "deliberate creation ....of a plant pathogen with increased virulence and host range" and does not also include such amnipulations concerned with animal pathogens.

5. Section I-E. Exemptions (p.33070, Col. 2). This entire Section is a very necessary and worthwhile addition to the Guidelines and Section I-E-5 certainly provides flexibility for making exemptions other than those now allowed pending adequate review and public input on potential recombinant DNA experiments or uses that pose little or no risk to public health or the environment. On the other hand, as mentioned below, I have specific criticisms about Section I-E-4 as now proposed which constitute one of the two major criticisms I have about the Proposed Revised Guidelines.

6. I will consider together my criticisms of Section I-E-4 (p.33070, Col. 2), Section III-A-1-b-(1) (p.33077, Col. 2), Section V-footnote 35 (p.33088, Col. 2), Appendix A (p.33089) and Appendix D to the Proposed Environmental Impact Statement (p.33158-33159).

The information provided to you by the RAC and others that led to the listing of organisms in Appendix A seems to have been misleading, inaccurate and incomplete and thus the statement on page 33044 "a list of donor-host pairs to be exempted... is a conservative one, restricted to pairs of organisms for which there is documented evidence of natural exchange" is false. In reviewing the information which is now in Appendix A to the Revised Guidelines and Appendix D to the Proposed Environmental Impact Statement (p.33158-33159) this past July, I wrote a lengthy letter to Drs. John Spizizen and Donald Helinski raising numerous issues pertaining to these lists. A copy of my letter to Drs. Spizizen and Helinski which was not dealt with in a substantial way at the August, 1978 Meeting of the RAC is attached to this letter as Appendix B. I will enumerate the problems as follows:

- a. The first problem concerns the absence of the specific criteria used to include or exclude groups of organisms from Appendix A. In footnote 35, Section V (p.33088, Col. 2), it defines genetic exchange "as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information." and then goes on to state, "Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section I-E-4." The difficulty arises in that nowhere in the Guidelines, whether in Section I-E-4 (p.33070, Col. 2) or in Appendix A (p.33089) or in Appendix D of the Proposed Environmental Impact Statement (p.33158-33159), are these more stringent standards for demonstration of exchange by natural physiological processes given. It is evident from the list of microorganisms in Appendix A and the existence of Section III-A-1-b-(1) (p.33077, Col. 2) that a more stringent set of standards for demonstrating exchange by natural physiological processes was sometimes but not always used. I should note that if the definition

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of genetic exchange as used in the Current Guidelines for Recombinant DNA Research were employed, then one list of exchangers would include any and all gram-negative microorganisms (see Appendix B to this letter). If, on the other hand, the criterion of genetic exchange by natural physiological processes is restricted to a demonstration of stable inheritance and expression of chromosomally transferred and integrated genetic material, which was alluded to as the definition being employed during discussions of this issue at the August, 1978 RAC Committee Meeting, then inclusion of all the organisms listed in Appendix A is unjustified (see Appendix B to this letter).

In establishing criteria and delineating the evidence necessary to list an organism or group of organisms in Appendix A, I think it realistic that relatedness be based on demonstration of genetic exchange and/or by demonstration of ~ 30-40% DNA sequence homology as revealed by DNA reassociation kinetics under stringent conditions. The necessity for demonstration of exchange by conjugation, transduction, or transformation is often a haphazard circumstance influenced by luck in the choice of strains, phages and/or growth conditions. It thus seems logical that use of current knowledge and techniques of molecular genetics to obtain information on relatedness should be permitted.

The basic idea for the exclusion stated in Section I-E-4 was due to the fact that recombinants formed in the laboratory by recombinant DNA technology between organisms that exchange DNA naturally would not be novel. The issue of safety was secondary. However, the safety issue has apparently been used to exclude some but not all organisms listed in Appendix D of the Proposed Environmental Impact Statement (p.33158) from the list of organisms in Appendix A of the Proposed Revised Guidelines (p.33089). In addition, the safety issue has been used inconsistently since those species of Bordetella, Pasteurella, Yersinia, Neisseria, etc. that are animal pathogens and which have been demonstrated to exchange plasmids, at least, with E. coli are excluded whereas all species of Pseudomonas and Erwinia, many of which are plant pathogens and many of which have not been demonstrated to exchange genetic information with E. coli, are included in Appendix A. I realize that NIH has greatest familiarity and responsibility for the public health of humans, but I should reiterate what I have often said that the human species is dependent upon plants, not the other way around. Thus while I am not totally opposed to the issue of safety being used to omit certain organisms from lists to be included in Appendix A, I do believe that the basis for such decisions should be stated and appropriate consideration to the safety of all important higher organisms should be given.

- b. The next problem concerns the taxonomic relatedness and evidence for genetic exchange among the organisms listed in Appendix A (p.33089). Since I am not an expert on bacterial taxonomy, I have relied on Bergey's Manual of Determinative Bacteriology (8th edition) and the guanine-cyto-

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sine contents of DNA in microorganisms as listed in the Handbook of Microbiology (Vol. II, p.585 and following) to provide some guidance. Since Appendix A lists bacterial genera on the list of exchangers with the exception of a few microorganisms that are also designated by species names, I list in Appendix C to this letter the ranges of DNA guanine-cytosine contents of the species within these genera as an indication of the potential relatedness among the microorganisms both within and between these various genera. As has been pointed out by others, there is DNA sequence homology and evidence of exchange of chromosomal genetic information among all members of the genera Escherichia, Shigella, Salmonella, Arizona, Citrobacter, Enterobacter, and Klebsiella, although the evidence for chromosomal gene exchange and degree of DNA sequence homology between either Klebsiella or Enterobacter and other members of the group are less substantial or convincing than evidence for the close relatedness among Escherichia, Citrobacter, Salmonella and Shigella. On the other hand, there are wide differences in DNA guanine-cytosine contents among the species that constitute the genera Serratia and Erwinia (including Pectobacterium) and although there is evidence for some Erwinia species of genetic exchange with Escherichia coli K-12, there are little or no data on exchange for most species in either of these genera. There is also inadequate DNA sequence homology and no data on chromosomal genetic exchange between either Serratia or Erwinia and members of the Enterobacteriaceae other than Escherichia coli.

The situation is worse in terms of the Pseudomonas species since guanine-cytosine contents vary from a low of 48% up to 69% and while there is evidence for chromosomal transfer using Inc P1 plasmids from only three or four species of Pseudomonas to Escherichia coli, no such evidence exists for any of the other species of Pseudomonas and certainly none with regard to genetic exchange between Pseudomonas species and other members of the Enterobacteriaceae such as Salmonella, Shigella, etc. One can make additional criticisms based on the information included in Appendix C to this letter but the available information really needs to be examined by an appropriately constituted panel of impartial experts.

- c. Another problem concerns the issue of whether data are available on reciprocity of exchange. For example, although there is evidence for transfer and inheritance of chromosomal information from Pseudomonas aeruginosa to E. coli K-12, I don't believe that the reciprocal experiment has been done. In any event, numerous other examples could be given since most studies have demonstrated transfer and inheritance to E. coli K-12 and not from E. coli. I believe it is logical to assume that if chromosomal material can be transferred and inherited in one direction that it could be inherited in the opposite direction. This assumption of reciprocity in the absence of data proving reciprocity should, however, be clearly stated.

- d. Another problem not considered concerns the fact that the exclusion granted in Section I-E-4 allows one to clone, for example, Salmonella DNA into Pseudomonas or vice versa even though there are no data to indicate that Salmonella species can exchange chromosomal genetic information with Pseudomonas species. In other words, the list of organisms in Appendix A (p.33089) is based almost entirely on the ability of organisms on the list to transfer chromosomal information to E. coli K-12. Thus, if it is the intent to conclude that if both organisms A and B can exchange chromosomal information with organism C that exchange between organisms A and B would occur even though this had not been demonstrated, this should be explicitly stated. I might add that such an assumption becomes somewhat farfetched when one considers that Acinetobacter calcoaceticus has a DNA guanine-cytosine content of 42-43% and another organism listed in Appendix A, Rhodopseudomonas sphaeroides, has a DNA guanine-cytosine content of 71%.
- e. Another problem with Appendix A is that it is incomplete. Lists of exchangers in the genera Bacillus, Streptococcus, Haemophilus and Streptomyces are lacking and these omissions will impede recombinant DNA research with these organisms and also the development of new host-vector systems. This is also bothersome since although it is now possible, for example, to clone Bacillus pumilis DNA in B. subtilis, the Proposed Revised Guidelines prohibit such an experiment until such time as the RAC approves certain B. subtilis derivatives as HV1 or resolves the problem in some other way.
7. Section II. Containment (p.33070-33076). Overall, this section is extremely well done. I very much support the idea of alternate practices of achieving physical containment at the P3 and P4 level and believe that the Laboratory Safety Monograph is extremely well done and will be most helpful and an important improvement over Appendix D of the Current NIH Guidelines. I do, however, have some minor points as enumerated below.
8. Section II-B-2-a-(13). dealing with laboratory gowns, coats or uniforms (p. 33072). Although it should be obvious that laboratory clothing is designed to protect undergarments from contamination with biological materials and that investigators wearing such protective clothing are most likely to experience aerosolized or spilled materials on their fronts, I have noted in laboratories that I have visited as well as in my own laboratory that investigators have a tendency to leave such laboratory coats open. I thus think it might be helpful to be more explicit in this section and to state that such laboratory gowns, coats or uniforms, etc. should be buttoned, zippered, etc.
9. Section II-B-2-b. Containment Equipment (p.33072). The use and necessity of biological safety cabinets in P2 laboratories is ambiguous. This section refers to aerosol-producing equipment but makes no mention of aerosol-producing procedures. It is also noteworthy that most of the equipment listed except for centrifuges is seldom used in recombinant DNA research. The most prevalent manipulations in laboratories using recombinant DNA technology with microorganisms are the growth of cul-

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tures which is usually by shaking and/or aeration and the processing of these cultures by centrifugation. It is also well known that substantial aerosols can arise by aeration of cultures during growth and similarly in the filling of centrifuge tubes, in the removal of supernatant fluids and in the suspension of sedimented cells. This is particularly true when using cultures and/or suspensions of one liter or more. Thus, even if centrifugation is done in sealed rotors or using safety centrifuge cups, it is still important to load and unload centrifuge rotors using a biological safety cabinet. There are also water baths and other means to preclude distribution of aerosols arising from aerated growth of microorganisms containing recombinant DNA. These issues should be clarified since some laboratories around the country operating under P2 containment do not have biological safety hoods and others do. Certainly if such hoods are not necessary, it will be a substantial saving of equipment monies that could be used for other purposes.

10. Section II-D. Biological Containment (p.33075). I noticed a few grammatical or typographical errors in this section (organelle DNA rather than organelle vector, O replaced by  $\lambda$  and conjugative plasmid rather than conjugation-proficient plasmid) but a few more substantial problems are enumerated below.

11. Section II-D-1. Levels of Biological Containment (p.33075). In this section, it states "Any combination of vector and host which are to provide biological containment must be constructed" (my underlining) which seems at variance with the statement in the Decision of the Director to Issue Revised Guidelines that wild-type organisms might be allowed as components of HV1 systems. The above wording would certainly seem to preclude the use of wild-type host-vector systems as would the requirement in Section II-D-2-b-(1) dealing with approval of HV1 systems wherein it states that an investigator must submit "data on any mutations which render this organism less able to survive or transmit genetic information". The intent of NIH should, therefore, be clarified. In resolving this issue, it should be noted that even prototrophic "wild-type" E. coli K-12 strains are not equivalent to wild-type E. coli strains recently isolated from nature since the K-12 strain has acquired mutations during its long maintenance under laboratory conditions causing LPS and K antigen syntheses to be defective. These laboratory-acquired mutations certainly contribute to biological containment. Thus in deciding on requirements for HV1 systems, one should not discount the importance of laboratory-acquired as opposed to induced mutations and should use the term "wild-type" in reference to the usual phenotype associated with strains of the species recently isolated from nature.

12. Section II-D-1-a-(1) EK1 (p.33075). Most host components of EK1 systems used in recombinant DNA research have mutations in addition to those acquired during K-12's laboratory sojourn that confer nutritional requirements, cause recombination to be defective, etc. and thus are not wild-type prototrophic strains of E. coli K-12. Indeed, it is known that certain of these mutations very much diminish survival in the intestinal tract or in other environments and/or reduce the likelihood for transmission of recombinant DNA. While the inclusion of such mutations in any newly developed host strains to be employed as components of EK1 systems should not be mandatory, it might be wise to encourage it. I make this suggestion because, during the International Congress of Microbiology Meeting in Munich this past week,

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I learned that 4 of 24 human volunteers who had been fed strains of E. coli K-12 in a risk assessment experiment being conducted in Great Britain excreted low titers of the E. coli K-12 strain for periods of up to four weeks. These results as well as those obtained by Rolf Freter (J. Inf. Dis. Vol. 137:624, 1978) in which prototrophic K-12 strains were found to persist in some animals under some conditions implies that the use of K-12 hosts without mutations to diminish their survival and/or ability to transmit DNA cloned on nonconjugative plasmid vectors will result in a higher cumulative amount of transfer of such recombinant plasmids to other microorganisms in nature than would occur if the K-12 hosts possessed mutations to diminish their survival and/or transmission ability.

13. Section II-D-1-c-(2) (p.33075). The requirement that "Reversion to host-independence must be less than  $1/10^8$  per vector genome per generation." is operationally difficult, if not impossible, to define since there is no meaningful operational definition of the term generation when applied to plasmids or to bacterial viruses. Quite possibly this should be clarified and put into some terminology of frequency starting from single plaques or cells containing plasmids.

14. Section II-D-2-a. Responsibility (p.33075). The statement that "HV1 systems other than E. coli K-12 and HV2 and HV3 host-vector systems may not be used unless they have been certified by the NIH", in view of the lists not contained in Appendix A, effectively precludes already approved recombinant DNA research with the B. subtilis and Saccharomyes cerevisiae cloning systems except for self cloning. It thus becomes important that NIH through the RAC begin to develop procedural means to inform the scientific community of exactly how to get approval of HV1 systems and to begin to develop appropriate guidelines for the performance of experiments that are permitted under the Current Guidelines but which will not be initially permitted under the Revised Guidelines.

15. Section III. Containment Guidelines for Covered Experiments (p.33076-33084). For the most part, I think that containment specifications are adequately justified by the available data on the increased safety of using E. coli K-12 host-vector systems but I do have a few minor points to make and one major reservation as detailed below. I should note that the statement on page 33058 (Col. 1) enumerating the criteria used to classify experiments is extremely good. One point which was omitted from the list concerns the potential consequences of transfer of the vector containing recombinant DNA to some other natural host or vector.

16. Section III-A-2-a. Viruses of Eukaryotes (p.33077-33080). There are numerous instances in which the stipulated containment is P3 + EK1 or P2 + an EK1 host and a vector certified for use in an EK2 system. The specified use of a vector certified for use in an EK2 system in the absence of its certified host is inconsistent with other sections of the Revised Guidelines which consider hosts and vectors as inseparable components of EK1 and EK2 systems. More importantly, this allowance permits wide differences in the actual level of biological containment dependent upon the selection of the vector that has been certified as a component of an EK2 system. All bacteriophage lambda vectors certified as components of EK2 systems possess mutations which cause their replication to be dependent on the propagating host, block lysogenization and cause death of all host cells infected. Indeed, the lambda vector constructed by Donoghue and Sharp meets EK2 standards irrespective

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of the propagating host. Although the lambda vectors constructed by the Blattner and Leder groups require use of either the DP50 or DP50 supF host, it is evident from the data provided by those laboratories and now substantiated by other investigators that the lambda vectors alone come very close to satisfying the level of containment required for EK2 certification all by themselves. Thus the use of a lambda vector that has been certified as a part of an EK2 host-vector system provides biological containment that would be equivalent to EK1.8 to EK2. On the contrary, with EK2 plasmid-host systems, almost all biological containment is provided by the host and little, if any, by the plasmid vectors which do not have mutations causing them to be dependent on the host. The plasmid vectors pMB9, pBR322 and pBR313 have defects which cause them to be mobilization-defective (Mob<sup>-</sup>) but this only decreases their probability for transmission 1000 to 10,000 fold when compared with the mobilization of unmodified Mob<sup>+</sup> ColE1-derived vectors. It should be noted that the Mob<sup>-</sup> defects of pMB9, pBR313 and pBR322 can be complemented by the ColK plasmid (Dogan et al, J. Inf. Dis. Vol. 137:676, 1978). It is also known that the low frequency of transmission of these plasmid vectors from χ1776 is also due to the conjugation defectiveness of χ1776, to the fact that χ1776 rapidly dies under nonpermissive conditions leading to its inability to transmit plasmid vectors and to the presence of a mutation causing a requirement for thymine or thymidine which leads to a marked reduction in the probability of transmission of plasmid vectors under nonpermissive conditions. These nonconjugative plasmid cloning vectors can also be transmitted from one microorganism to another by generalized transduction and this is diminished substantially in χ1776 which is partially or totally resistant to the generalized transducing phages P1, D108, Mu, etc. and which possesses a thyA mutation that reduces transductional transmission of plasmid vectors under nonpermissive conditions. I should indicate that James Robeson in our laboratory has shown that the generalized transducing phage, P1, can infect 70% of over 500 wild-type strains of E. coli tested and there is a substantial amount of literature to indicate that P1 has a very broad host range and is able to infect strains of Salmonella, Shigella, Klebsiella, etc. These results suggest that transductional transmission of nonconjugative plasmid vectors (which are not dependent on their propagating host) may contribute to plasmid transmission. It is therefore evident that the use of plasmid vectors that have been certified as components of EK2 plasmid-host systems provide only nominal biological containment compared to the use of lambda vectors which have been certified as components of EK2 phage-host systems. I should also point out that the reference to these plasmid vectors that have been certified as components of EK2 host-vector systems as "non-mobilizable" in Appendix F to the Proposed Environmental Impact Statement (p.33168) is erroneous. It is thus evident that experiments classified in Section III-A-2-a as requiring either P3 + EK1 or P2 + an EK1 host and a vector certified for use in an EK2 system can actually be done under P2 + EK1.1 if the investigator chooses to use a plasmid vector certified as a component of an EK2 host-vector system. Furthermore, the actual level of biological containment might even be less if the investigator selected an E. coli K-12 host like some of those used in the feeding experiments mentioned above.

Although the consequences of transmission of vectors containing viral DNA to other microorganisms is not discussed in Appendix E or F to the Proposed Environmental Impact Statement (p.33159-33169), Dr. Wallace Rowe has informed me that some consideration was given to this issue. Although I concur that working with viral DNA

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inserts in E. coli K-12 is probably safer than working with the intact infectious virus, I do have reservations based on the lack of appreciation by the various groups that studied and reviewed the cloning of viral DNA in E. coli of the differences between phage and nonconjugative plasmid vectors and the consequences of transmission of recombinant DNA to wild-type microorganisms in nature. The recent results of experiments with polyoma in E. coli K-12, conducted by Dr. Michael Fried in Great Britain and by Drs. Malcolm Martin, Wallace Rowe and their colleagues in the U.S., also contribute to my concern. As you will recall at the Falmouth Workshop, while it was the consensus that one could not likely convert E. coli K-12 into a pathogen, much less an epidemic pathogen, there was considerable concern and debate about the consequences of transmission of vectors containing recombinant DNA to microorganisms indigenous to various natural habitats. I am also informed that certain virally specified mRNAs need not be processed to lead to synthesis of fully functional viral proteins and that some virally-specified proteins can be made using E. coli RNA polymerase with an E. coli generated translation system. I thus believe that this section of the Revised Guidelines dealing with the cloning of viral DNA in E. coli K-12 host-vectors needs to be examined not only by virologists but by some of the types of experts who were in attendance at the Falmouth Workshop Meeting.

In view of what I have read and learned and based on some of the preceding comments, I would not have voted in favor of lowering the containment levels for cloning viral genetic information in E. coli K-12 to the levels now stipulated in the Revised Guidelines if I were still a member of the RAC. This is particularly true in view of the fact that the risk assessment experiments which were to have defined what levels of containment are fitting have yet to be completed.

17. Section III-B-2. Return of DNA segments to non-HV1 host of origin. (p.33080). The provisions of containment for a prokaryote which does not exchange genetic information with E. coli, the so-called Host B, are poorly thought out and in need of refinement. For example, if Host B is a Class 1 agent, the cloning into E. coli would require P2 + EK1 or P1 + EK2 and the return of the potential double vector to Host B would only require P1 containment. If Host B were a Class 2 agent, and this is not precluded, then the nonsymmetry of the containment required and the problems associated with double vectors become even more exaggerated. Obviously, these points need to be addressed and further refinement of this section is needed. Based on comments in the Decision of the Director to Issue Revised Guidelines statement, I surmise that it was intended to use the cloning in E. coli K-12 to purify a genomic segment from Host B which would then be separated from the E. coli vector, purified and returned to Host B in the absence of the E. coli vector. I should add that it might be wise to note in this section that P2 containment would be advisable if Host B is a Class 2 agent or a plant pathogen.

18. Section III-C-5. Fungal or Similar Lower Eukaryotic Host-Vector Systems (p.33084). The stipulations for cloning DNA from a non-HV1 host into E. coli K-12 and return to that host (designated Host C) result in the same problems detailed in the immediately preceding comment.

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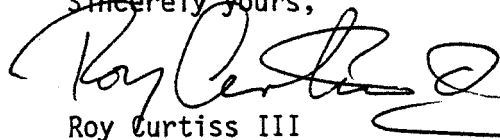
19. Section IV. Roles and Responsibilities (p.33084-33087). I believe this section has been thoroughly thought out and clearly written. I wholeheartedly support the concept that an institution receiving NIH funds should be responsible for adherence to the Guidelines even when recombinant DNA research at that institution is not necessarily supported by NIH funds. I also believe that greater reliance needs to be placed on the Institutional Biosafety Committee and the proposals contained in Section IV will both better facilitate the research and result in better adherence to the Guidelines because of greater local responsibility. I also think that the insistence on a biological safety officer for institutions conducting P3 and P4 level research is a wise decision which I fully support.

20. Section V. Footnotes and References (p.33087-33089). Most comments I had pertaining to this section have been given above. I should note in reference to footnote 1 and Appendix B to the Proposed Revised Guidelines (p.33089-33090) that the current listing of etiologic agents is incomplete and in some respects inaccurate. For example, under fungal agents that are Class 2 agents, one finds Actinomycetes which first of all are bacteria, not fungi. Furthermore, the term "Actinomycetes" is a group designation which includes among its eight families the genera Actinomyces, Nocardia, Streptomyces and Mycobacterium and all of these are then classified as Class 2 agents. Since most species of Streptomyces and many of Nocardia and Mycobacterium are harmless soil bacteria, I hope the revision of the list of etiologic agents will rectify these problems and errors. I might also note that Rickettsia and Chlamydia are obligate intracellular bacterial parasites and not viral agents and should thus be classified under the bacteria.

21. In view of my comments concerning biological containment of the E. coli K-12 host-vector systems and the large difference between the containment, and in some instances utility, provided by EK1 vs EK2 systems, it might be worthwhile for NIH to reconsider Dr. Wacław Szybalski's suggestion of several years ago to specify EK1.5 systems. In this regard, we have a reasonable number of well characterized strains already in our collection that should, in conjunction with nonconjugative plasmid vectors, satisfy anticipated requirements for such an EK1.5 level of biological containment.

Although my comments have been numerous and lengthy, I wish to reiterate my enthusiastic support for adoption of the Proposed Revised Guidelines for Recombinant DNA Research following refinement and/or reconsideration based on comments I and others have made. If I can provide any additional information or advice, I would be pleased to do so.

Sincerely yours,



Roy Curtiss III

RCIII/pp  
Enclosures